

Haematological Parameters and Behavioural Responses of *Clarias gariepinus* Exposed to Sub-Acute *Senna occidentalis* Ethanol Leaf Extract

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Abstract The health of fish largely depends on the quality of their blood, which is relevant in indicating the state of the fish's health. The present study was conducted to evaluate the effects of sub-acute exposure to *Senna occidentalis* ethanol leaf extract on the haematological parameters and behavioural responses of *Clarias gariepinus* juveniles. The study was carried out at the Fish Hatchery Complex, FUNAAB. One hundred and eight (108) juveniles of *Clarias gariepinus* with an initial average body weight of 16 ± 2 grams and length of 8.5 ± 0.5 cm were used. The leaves of *Senna occidentalis* were obtained at Old Bola Ahmed Tinubu Road, off Iju Road, Ifako-Ijaiye LGA, Lagos State. The experiment had four treatments and three replicates, each with 9 fish per treatment tank. During the 23-day subacute toxicity test, *C. gariepinus* exposed to graded doses of *Senna occidentalis* were closely observed for behavioural responses. Haematological parameters such as Red Blood Cell, White Blood Cell, Packed Cell Volume, Haemoglobin, and White Blood Cell Differential Counts were also carried out. Analysis of variance was used to assess the data. The results showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell ($3.30-2.13 \times 10^{12/L}$), White Blood Cell ($15.82-10.90 \times 10^9/L$), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *Senna occidentalis* concentrations increased in relation to the control treatment. These findings provide a baseline that what is natural may not be automatically safe, as the results of this research showed poor growth and fish deaths. These findings indicate that *Senna occidentalis* ethanol leaf extract may pose sub-acute toxic effects to *Clarias gariepinus*, and the results may provide useful baseline information for fish health assessment and the safe use of medicinal plant extracts in aquaculture environments.

Keywords *Clarias gariepinus*; *Senna occidentalis*; Haematology; Behavioural response; subacute toxicity; Plant extract

1 Introduction

Aquatic ecosystems are fundamental to global biodiversity, food security, and ecological stability; however, they are increasingly compromised by contamination from a broad spectrum of anthropogenic substances (Thanigaivel et al., 2023). These environmental pollutants enter the aquatic systems through runoffs and discharges (Amoatey and Baawain, 2019; Das et al., 2024) and have been widely reported to disrupt multiple physiological systems in fish (Mustafa et al., 2024) and biochemical processes (Rocha et al., 2018). Their impact includes impairments to immune balance, reproduction, and metabolism, with effects often under concurrent environmental stressors such as hypoxia and pH fluctuations.

Recent studies have increasingly examined environmental pollutants and their effects in aquatic environments (Adeleye et al., 2024; Sefali et al., 2026). While most of this research is centered on conventional contaminants such as heavy metals, pesticides, and pharmaceuticals, comparatively less research has been done on plant-derived bioactive compounds.

Plants and their derivatives serve as key sources of nutrients for humans and animals while also providing medicinal benefits (Diouf et al., 2019; Samtiya et al., 2020). They contain bioactive compounds whose effects are either beneficial or harmful, and are largely concentration-dependent (Ali et al., 2022; Dey et al., 2022). *Senna occidentalis*, a medicinal plant widely used in traditional systems and reported to possess potent bioactive constituents, warrants toxicological evaluation in aquatic organisms such as *Clarias gariepinus*. Egharevba et al. (2010) stated that *Senna occidentalis* L. Link (Leguminosae), formerly known as *Cassia occidentalis* L., is well-known for its wide range of medical applications and is utilized locally to treat various human and animal illnesses. However, *Senna species* seeds, leaves, and roots have been shown to have a variety of toxicities despite their enormous medicinal potential (Gebrelibanos et al., 2014). In other words, exposure to some of these plants may be toxic to humans and animals, even with the wide range of medicinal potentials exhibited by many botanical products (plants) (Belay and Enyew, 2016).

Hematological analysis of peripheral blood parameters and quantitative assessment of blood cell morphology serve as practical, cost-effective tools in fish toxicology (Witeska et al., 2023). Accordingly, the present study evaluated erythrocyte counts, leukocyte differentials, hemoglobin concentration, etc., in *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract to assess hematotoxic potential. Packed cell volume (PCV) and haemoglobin concentration are standard indicators of anaemia in aquaculture (Afia and Gift, 2017), while RBC indices like MCHC, MCH, and MCV aid diagnosis (Iheanacho et al., 2017). Hematological responses of fish to xenobiotics vary with the toxicant, exposure time, and biological factors such as species, age, and size (Ahmed et al., 2020), and may represent adaptation, damage, or both (Witeska et al., 2023). Given that plant-derived bioactive compounds can act as xenobiotics in aquatic systems, these haematological parameters, alongside behavioral responses, are therefore critical for evaluating sub-acute toxic effects of *Senna occidentalis* ethanol leaf extract on *Clarias gariepinus*.

2 Materials and Methods

2.1 Experimental site

The study was conducted at the Fish Hatchery Complex, Aquaculture and Fisheries Management Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (FUNAAB).

2.2 Collection of juveniles of *Clarias gariepinus*

A total of one hundred and eight (108) juveniles of *Clarias gariepinus* with an initial average body weight of 16 ± 2 grams and length of 8.5 ± 0.5 cm were obtained from Path Farm, Sagamu, Remo, Ogun State, Nigeria, and transported in a 50-liter keg to the Fish Hatchery, FUNAAB.

2.3 Acclimatisation of the *C. gariepinus* juveniles

The *C. gariepinus* juveniles were acclimatised for two weeks; in that period, they were fed 1.8 mm imported Skretting feed, which contained 45% crude protein, twice a day, in the early morning and in the evening. Feeding was stopped 48 hours before the beginning of the experiment, and wastes and wasted feed were taken out daily along with replenishing water.

2.4 Collection and preparation of *Senna occidentalis* leaves

The leaves of *Senna occidentalis* were obtained from Old Bola Ahmed Tinubu Road, off Iju Road, Ifako-Ijaiye LGA, Lagos State, Nigeria, and authenticated by the Forestry and Wildlife Management Department, FUNAAB. Fresh leaves of *S. occidentalis* were air-dried for two weeks and ground using a Binatone BLS-360 1.5 L electronic blender; then 300 g of the powdered leaves were obtained and soaked in 1400 mL of ethanol and 600 mL of distilled water (70% ethanol); the solution was stirred continuously at intervals for 72 hr (Jun et al., 2012). The solution was filtered, subjected to a rotary evaporator (50 °C), and oven-dried for 24 hr at 40 °C using a low-temperature oven drier to achieve a more concentrated extract at the Lagos University Teaching Hospital (LUTH). The extract was then stored in a refrigerator using an airtight container.

2.5 Experimental design and procedure

The experiment had four treatments and three replicates, each with 9 fish per treatment tank. From the acclimatised fish, 108 juvenile catfish were randomly distributed into 12 plastic tanks with a capacity of 35 litres, each filled with 10 litres of water for the experiment. A completely randomised design was adopted. From the stored extracts, 3000 mg/25 cL, 5000 mg/25 cL, and 7000 mg/25 cl of distilled water were used as a stock solution, from which 5 ml of each concentration was infused daily into the plastic tanks.

2.6 Behavioural studies

During the 23-day sub-acute toxicity test, *C. gariepinus* exposed to graded concentrations of *S. occidentalis* was closely observed for the subsequent behavioural changes: air gulping, stunned positioning, skin peeling, aggression, and erratic swimming (fast and spiral movement). Weak, moderate, and high rankings were given to the observed changes.

2.7 Haematological test

According to Erhunmwunse and Ainerua (2013), haematological parameters, including red and white blood cell counts, packed cell volume, haemoglobin, and white blood cell differential counts, were performed following standard procedures.

2.7.1 Determination of haemoglobin

Using the Randox kit, haemoglobin was measured spectrophotometrically. Potassium ferricyanide (0.61 mmol/L), potassium cyanide (0.77 mmol/L), potassium phosphate (1.03 mmol/L), and 0.1% v/v surfactant are all present in the reagent.

Procedure:

The test tubes were labelled as blank, standard, and tests. 20 µL of whole blood was added to each tube. Additionally, 5 mL of the reagent was added to each tube, which was then incubated at room temperature for 3 minutes. The absorbance of all tubes was read at 540 nm against the reagent blank.

Haemoglobin conc. (g/dL) = Abs of sample X 36.77 --- (1)

2.7.2 PCV (Packed Cell Volume)

Blood was poured into a simple capillary tube until it was about $\frac{3}{4}$ full. Plasticine was used to seal the tube's open end. The sealed tube was centrifuged at exactly 12,000 revolutions per minute for five minutes in a Hawksley micro-hematocrit centrifuge. The packed cell volume value was determined and expressed as a percentage for each tube after placing it in a micro hematocrit reader.

2.7.3 RBC (Red Blood Cell count)

The hemocytometer was used to determine the red blood cell count. Blood was diluted 1:200 with red blood cell diluting fluid using a red blood cell pipette. After mixing the dilution and waiting two minutes, the hemocytometer's counting chamber was filled, and the red blood cells were counted using a 40x microscope objective. The total number of counted cells was expressed in cubic millimetres or litres and multiplied by 10,000.

2.7.4 Total WBC (White Blood Cell Count)

The haemocytometer's white blood cell pipette was used to dilute the blood 1:20 with WBC diluting fluid. The liquid was slowly combined with the blood. The dilution was added to the counting chamber, and the WBCs were counted using a microscope's x10 objective. The total cell count was expressed in millilitres or litres and then multiplied by 50.

2.7.5 WBC differential count

A drop of blood was evenly distributed across a clean, grease-free slide to create a thin blood film using a smooth-edged spreader. After staining the blood film with aqueous stains, it was fixed in acetone-free methyl alcohol for approximately three to five minutes to prevent hemolysis upon contact with water. It was then left to dry. After applying the field, A and B stains to the blood film, 100 white blood cells were separated under a microscope with oil immersion objectives.

2.8 Statistical analysis

Data obtained from the experiments were collated and subjected to analysis of variance using SPSS version 20.0, with significance set at $p < 0.05$. Duncan's Multiple Range Test (DMRT) was used for comparison tests.

3 Results

3.1 Behavioural signs

The behavioural and morphological responses of *Clarias gariepinus* exposed to varying concentrations of *S. occidentalis* ethanolic extract are presented in Table 1.

Table 1 Results of behavioural responses

Behavioural response	Control 0 mg/ 25 cL	Treatment A 3000 mg/25 cL	Treatment B 5000 mg/25 cL	Treatment C 7000 mg/25 cL
Fin deformation	-	+	+++	+++++
Barbel whitening	-	+++	+++	+++++
Air gulping	-	+	+++++	+++++
Stunned positioning	-	+	+++	+++
Aggression	-	-	+++	+++
Erratic swimming	-	+	+	+++
Loss of balance	-	-	+	+++

-: Normal; +: weak; +++: moderate; +++++: high

Throughout the experiment, behavioural and morphological changes in the test fish were noted at 12-hour intervals. While there was a slight whitening of the barbels in the fish in the treatment tanks, the treatment tanks showed distinct morphological and behavioural changes. For instance, the fish in treatment one showed modest alterations in the shape and orientation of their fins, and their barbels lightened somewhat. In contrast, the fish in treatment two gulped due to insufficient dissolved oxygen, turned hostile, displayed a stunned posture, and showed mild fin deformation. The fish in treatment three exhibited moderate stunned positioning, aggression, erratic swimming, loss of body balance, and a high degree of fin deformation, barbel whitening, and gill damage due to insufficient dissolved oxygen.

3.2 Results of haematological parameters

According to Iheanacho et al. (2017), haematological indices are vital health markers that show the condition of fish's health both before and after trials.

Table 2. showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell ($3.30-2.13 \times 10^{12/L}$), White Blood Cell ($15.82-10.90 \times 10^9/L$), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *S. occidentalis* concentrations increased relative to the control treatment, while Heterophile (28.02%-37.04%) increased as *S. occidentalis* concentrations increased.

4 Discussion

Hematological parameters serve as standard indicators of fish health under aquaculture conditions and in ecotoxicological studies (Witeska et al., 2022). Their diagnostic value was demonstrated by Bojarski et al. (2022), who found hematological indices to be the most sensitive biomarkers of toxicity in *Cyprinus carpio*. These parameters are sensitive and are indicators of physiological alterations in fish; they provide extensive information on oxygen transport, immune status, stress, cytotoxicity, and genotoxicity (Witeska et al., 2023). In the present study, *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract exhibited significant alterations in white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hb), packed cell volume (PCV), and erythrocyte indices (MCV, MCH, and MCHC), indicating that phytochemicals from *S. occidentalis* can disrupt hematological homeostasis. This supports hematology as a practical biomarker for evaluating plant-based xenobiotics in aquaculture species under laboratory conditions.

Table 2 Results of haematological parameters

Parameters	Control	Treatment A	Treatment B	Treatment C
Conc.	0 mg/25 cL	(3000 mg/25 cL)	(5000 mg/25 cL)	(7000 mg/25 cL)
PCV (%)	38.31 ^c	34.22 ^b	28.23 ^a	28.11 ^a
Hb (g/dL)	12.31 ^d	11.82 ^c	9.71 ^a	9.50 ^a
RBCs (10 ^{12/L})	3.30 ^b	3.02 ^b	2.51 ^a	2.13 ^a
WBCs (×10 ^{9/L})	15.82 ^e	14.11 ^d	13.75 ^c	10.90 ^a
HET. (%)	28.02 ^a	34.12 ^c	34.02 ^c	37.04 ^d
LYM (%)	69.03 ^c	64.03 ^b	65.10 ^b	60.11 ^a
MCV (pg)	115.15 ^c	133.33 ^b	112.80 ^a	112.33 ^b
MCH (pg)	45.24 ^d	39.33 ^c	38.80 ^b	37.27 ^a
MCHC (g/dL)	34.71 ^c	34.64 ^c	33.93 ^b	32.27 ^a

Means along each row with different superscripts are significantly ($p < 0.05$) different. The values shown are the means and the standard deviations. Conc.= concentration, PCV = packed cell volume, Hb = haemoglobin, RBCs= red blood cell counts, WBCs =white blood cell count, HET = heterophil, LYM = lymphocyte, MCH = mean cell haemoglobin, MCV = mean cell volume, MCHC = mean cell haemoglobin concentration, fL = femtolitre, pg = picogram

Gebrelibanos et al. (2014), reported that *Senna* species had been known to cause a variety of toxicities despite their many potential medicinal benefits; this has been a major concern in aquaculture production (Idowu et al., 2017), as well as a condition wherein living organisms exhibit changes in their bodily systems and manifest symptoms due to impaired physiological functions (Adedeji et al., 2017), such as aggression, loss of balance, and erratic swimming. During this experiment, it was frequently noticed that the fish responded to environmental changes as soon as the extract was added to the water body. These responses included the morphological and behavioural indicators shown in Table 1. Fish were seen gasping for breath in the tanks containing high concentrations of *S. occidentalis* ethanol leaf extract (5000 and 7000 mg/ 25 cL), and whitening of the fins and barbels were noted in the tanks with concentrations of 3000 and 7000 mg/ 25 cL; this could be due to the potency and toxicological effectiveness of the phytochemicals (alkaloids, flavonoids, tannins, glycosides, steroids, and saponin) found in the plant extracts.

The gradual changes in the haematological parameters of *C. gariepinus* juveniles recorded in this study indicate that *S. occidentalis* ethanol leaf extract affects the blood of the exposed fish. The findings showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell (3.30-2.13 x 10^{12/L}), White Blood Cell (15.82-10.90 x 10^{9/L}), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *S. occidentalis* concentrations increased relative to the control treatment, while Heterophils (28.02%-37.04%) increased as *S. occidentalis* concentrations increased; this could be as a result of the poisonous potentials of *S. occidentalis* ethanolic extract, which rose with an increase in extract concentration in *C. gariepinus* blood; this is in line with studies by Eriegha et al., (2017); Idowu et al., (2020), who confirmed that infected fish had lower PCV values than healthy fish and observed a similar pattern in fish exposed to toxicants. Erythrocytes serve as models for assessing toxicity-induced apoptosis, oxidative stress, and cellular damage in fish (Sakuragui et al., 2019). Thus, the altered erythrocyte morphology and indices recorded here in *C. gariepinus* indicate that *S. occidentalis* phytochemicals may promote oxidative injury or apoptotic pathways in circulating red blood cells. Moreover, a decrease in RBC, Hb, and PCV typically indicates an anemic response to toxicants, resulting from direct hemolysis. A similar decrease in Ht, RBC, and Hb was reported in *Anabas testudineus* exposed to acrylamide (Ligina et al., 2022). Also, Ko et al. (2019) reported a concentration-related decrease in Ht, Hb, and RBC of *Platichthys stellatus* intoxicated with hexavalent chromium.

Leukocyte count (WBC) and differential leukocyte count (DLC) are standard indicators of immune status in fish. Toxicants commonly alter WBC, producing leukocytosis or leukopenia (Witeska et al., 2023). In the present study, *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract showed a decrease in the value of WBC, suggesting that phytochemicals from *S. occidentalis* elicit a cytotoxic suppression of leukocyte homeostasis. Thus, hematology provides sensitive, practical biomarkers for assessing plant-based toxicants and indiscriminate use of *S. occidentalis* in aquaculture.

5 Conclusion

It can be concluded that haematological parameters are reliable and useful indicators of fish health status in response to environmental changes. The present study demonstrated that sub-lethal concentrations of *Senna occidentalis* leaf extract disrupt hematological homeostasis in *Clarias gariepinus*. Significant reductions in RBC, Hb, and PCV with altered MCV indicate hemolytic or hypoxic anemia. These changes are consistent with oxidative injury, impaired erythropoiesis, and cytotoxicity reported for other xenobiotics, indicating that bioactive compounds in *S. occidentalis* exert hematotoxic effects. Therefore, it can be stated that *S. occidentalis* toxicity caused consistent, gradual damage to the immune system of *C. gariepinus*. In relation to the potential risk of using *Senna occidentalis* in aquaculture environments or its relevance for toxicological assessment in fish, these data offer a reference point for assessing fish health and promoting safe application of plant-based extracts in aquaculture.

Author's Contribution

Idowu Adekunle Adedoyin conceived and designed the study, critically reviewed the manuscript, and approved the final version. Adesanya Oluwatosin Emmanuel participated in the hatchery experiment, drafted the manuscript, and contributed to funding support. Towolawi Adeleke Taofik contributed to funding and conducted the haematological analyses together with Adesanya Oluwatosin Emmanuel. Odukoya Abimbola Erastus documented the sub-acute exposure experiment and also contributed to manuscript preparation. Adekola Mukaila B. supervised the overall study, participated in the experimental design and coordination, and assisted in drafting the manuscript. All authors read and approved the final manuscript.

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